DATA EVALUATION RECORD

1. **CHEMICAL:** Thiophanate-methyl.
Shaughnessey No. 102001.

2. **TEST MATERIAL:** 1) Thiophanate-methyl (Topsin M); Lot No. TIF-01016; 97.57% active ingredient; an off-white powder. 2) ^{14}C-Thiophanate-methyl; Lot No. Amersham CFQ.4519GTW/C2/52; radiopurity of 95.3%, and chemical purity of 90.1%.

3. **STUDY TYPE:** Growth and Reproduction of Aquatic Plants - Tier 2. Species Tested: *Navicula pelliculosa*.

4. **CITATION:** Hoberg, J.R. 1992. Thiophanate-methyl - Toxicity to the Freshwater Diatom *Navicula pelliculosa*. Report No. 91-10-3965. Conducted by Springborn Laboratories, Inc., Wareham, MA. Submitted by Atochem North America, Inc., Philadelphia, PA. EPA MRID No. 422298-02.

5. **REVIEWED BY:**

Heather Mansfield, Zoologist, Section 2
Ecological Effects Branch
Environmental Fate and Effects Division

Signature: Heather Mansfield 3/29/93

6. **APPROVED BY:**

Norman J. Cook, Head, Section 2
Ecological Effects Branch
Environmental Fate and Effects Division

Signature: Norman J. Cook 04-20-93

7. **CONCLUSIONS:** This study is scientifically sound and meets the requirements for a Tier 2 aquatic plant growth and reproduction study. Based on mean measured concentrations, the NOEC, LOEC, and EC_{50} for *N. pelliculosa* exposed to thiophanate-methyl were 0.43, 0.89, and 0.93 mg ai/l, respectively.

8. **RECOMMENDATIONS:** N/A.

9. **BACKGROUND:**

10. **DISCUSSION OF INDIVIDUAL TESTS:** N/A.

11. **MATERIALS AND METHODS:**

A. **Test Species:** The diatom used in the test, *Navicula pelliculosa*, came from laboratory stock cultures originally obtained from Carolina Biological Supply Company, Burlington, NC. Stock cultures were maintained in Algal Assay Procedure (AAP) medium (Table 1, attached) under test conditions. Transfers were made to fresh medium once weekly. The culture used as the inoculum for the test was transferred to fresh medium two days before test initiation.

B. **Test System:** Test vessels were sterile 125-ml flasks fitted with stainless steel caps which permitted gas exchange. The vessels were conditioned by rinsing with appropriate test solutions and 50 ml of the test or control solution were placed into each flask. The test medium was the same as that used for culturing with a pH of 7.5. Test vessels were maintained on an orbital shaker (shaking rate of 100 rpm) under continuous cool-white illumination (approximately 350-475 footcandles at the surface of the media) in an environmental chamber. The temperature in the chamber was maintained at 25°C.

C. **Dosage:** Five-day growth and reproduction test. Based on the results of preliminary tests, five nominal concentrations of 0.12, 0.25, 0.50, 1.0, and 2.0 mg active ingredient (ai)/l were selected for the definitive test.

A 30 µl aliquot (in acetone) of a 0.455 mg ai/ml ^{14}C-thiophanate-methyl solution was added to 1000 ml of 1.99 mg ai/l unlabeled thiophanate-methyl solution (in AAP medium) to which an additional 70 µl of acetone had been added. This solution was equal to the highest test solution (2.0 mg ai/l) concentration. Lower concentration test solutions were created by addition of appropriate volumes of the highest test solution to nutrient medium. A medium and solvent (0.1 ml acetone/l) control were also prepared.

D. **Test Design:** The test consisted of 3 replicate flasks per treatment level and controls. An inoculum of *Navicula pelliculosa* cells calculated to provide 10,000

cells/ml was aseptically introduced into each flask within twenty minutes of solution addition. The inoculum volume was 1 ml per flask. At each 24-hour interval, cell counts were conducted on each replicate vessel using a hemacytometer and compound microscope. On day 5 of the test, the cells were sonicated for five minutes prior to counting.

The conductivity and pH were measured at test initiation and termination. Temperature was recorded continuously with a minimum/maximum thermometer. The shaking rate of the orbit shaker was recorded daily. The light intensity was measured at the beginning of the test and every 24-hour interval of the exposure period.

At test initiation and termination, samples were removed from each test solution and the controls for analysis by liquid scintillation counting. Three quality control (QC) samples were prepared at test initiation to judge the precision of the analyses.

E. **Statistics:** Negative and solvent control data were pooled if no significant differences were determined by a t-test. If the controls were significantly different, comparisons were made against the solvent control data. For each observation period, the EC_{10}, EC_{50}, and EC_{90} values and their 95% confidence intervals (C.I.) were determined by linear regression of response (percent reduction of cell density as compared with the control) vs. mean measured concentration over the range of test concentrations. Various mathematical manipulations (logarithm and probit transformations) were used on the concentration and response data to get the linear regression with the highest coefficient of determination (R^2).

The no-observed-effect concentration (NOEC) was determined using one-way analysis of variance (ANOVA) coupled with Bonferroni's test. The data were checked for normality using the Chi-square test and for homogeneity of variance using Hartley's test before conducting ANOVA.

12. **REPORTED RESULTS:** The mean measured concentrations of the test solutions were 0.11, 0.22, 0.43, 0.89, and 1.70 mg ai/l which averaged 87% of nominal concentrations (Table 2, attached).

Cell densities determined at each observation time are presented in Table 3 (attached). At test termination, mean cell densities ranged from 20 to 59 x 10^4 cells per ml. Cell number in the two highest treatment concentrations was significantly reduced in comparison to the pooled control and the NOEC was therefore determined to be 0.43 mg ai/l. At test termination, cells in the two highest treatment groups appeared to be slightly bloated, with cell fragments present. A few slightly bloated cells were observed in the 0.43 mg ai/l treatment groups. The cells exposed to the two lowest concentrations appeared to be healthy. The 120-hour EC_{50} was determined to be 0.95 mg ai/l with a 95% C.I. of 0.37-2.8 mg ai/l.

Conductivity ranged from 110 to 200 μmhos/cm. The pH was 7.4-7.5 in all test solutions and the controls at test initiation and 7.5-8.5 at termination. The temperature remained at 25°C during the study.

13. **STUDY AUTHOR'S CONCLUSIONS/QUALITY ASSURANCE MEASURES:** No conclusions other than those stated above were made by the study author.

The study director confirmed that this study was conducted in compliance with Good Laboratory Practice (GLP) regulations (40 CFR Part 160) with the exception that stability, characterization, and verification of test substance identity and maintenance of records on the test substance are the responsibility of the test sponsor. Additionally, routine water analyses were conducted by an independent laboratory that did not operate under GLPs. A statement indicating adherence to Quality Assurance was enclosed in the report.

14. **REVIEWER'S DISCUSSION AND INTERPRETATION OF STUDY RESULTS:**

A. **Test Procedure:** The test procedure and the report were generally in accordance with the SEP and Subdivision J guidelines, except for the following:

The light intensity during the test [3780-5130 lux (1 footcandle = 10.8 lux)] was higher or lower than recommended (4300 lux).

B. **Statistical Analysis:** The reviewer used EPA's Toxanal program to determine the EC_{50}. By employing the moving

average angle method and comparing the treatment cell densities to those of the pooled control, a slightly more conservative EC_{50} and confidence interval were determined (see attached printout). The 5-day EC_{50} and its associated 95% C.I. were 0.93 mg ai/l and 0.83-1.06 mg ai/l. The reviewer used ANOVA coupled with Dunnett's test to determine the lowest-observed-effect concentration (LOEC) and NOEC in comparison to the pooled control data (see attached printout). The results are based on cell density and mean measured concentration data. The results from the analysis indicated that the LOEC was .89 mg ai/l and the NOEC was 0.43 mg ai/l. Bloated cells were observed at 0.43 mg ai/l, but this level was not statistically different from the control.

C. **Discussion/Results:** This study is scientifically sound and meets the requirements for a Tier 2 aquatic plant growth and reproduction study. The EC_{50} for *N. pelliculosa* exposed to thiophanate-methyl was 0.93 mg ai/l based on mean measured concentrations. The NOEC and LOEC were 0.43 and 0.89 mg ai/l, respectively.

D. **Adequacy of the Study:**

(1) Classification: Core.

(2) Rationale: N/A.

(3) Repairability: N/A.

15. **COMPLETION OF ONE-LINER:** Yes, 3/29/93.

Table 1. **Composition of algal growth medium (AAP Medium) used in this study.**

Compound	Concentration (mg/L)
$NaNO_3$	25.5 mg/L
$MgCl_2 \cdot 6H_2O$	12.164 mg/L
$CaCl_2 \cdot 2H_2O$	4.41 mg/L
$MgSO_4 \cdot 7H_2O$	14.7 mg/L
K_2HPO_4	1.044 mg/L
$NaHCO_3$	15.0 mg/L
H_3BO_3	185.52 µg/L
$MnCl_2 \cdot 4H_2O$	415.61 µg/L
$ZnCl_2$	3.271 µg/L
$CoCl_2 \cdot 6H_2O$	1.428 µg/L
$CuCl_2 \cdot 2H_2O$	0.012 µg/L
$Na_2MoO_4 \cdot 2H_2O$	7.26 µg/L
$FeCl_3 \cdot 6H_2O$	160.0 µg/L
$Na_2SiO_3 \cdot 9H_2O$[a]	101.0 mg/L
$Na_2EDTA \cdot 2H_2O$	300.0 µg/L

[a] Included in medium for diatoms only.

Source: W.E. Miller, J.C. Greene and T. Shiroyama. 1987. The *Selenastrum capricornutum* Printz algal assay bottle test. EPA 600/9-78-018. U.S. Environmental Protection Agency, Washington, D.C.

Table 2. **Measured concentrations of Thiophanate-methyl used in the 120-hour toxicity test with *Navicula pelliculosa*.**

Nominal Concentration (mg A.I./L)		Measured Concentration (mg A.I./L) 0-Hour	120-Hour	Mean[a]
2.0	A	1.7	1.8	1.7(<0.1)
	B	1.7	1.7	
	C	1.7	1.7	
1.0	A	0.88	0.87	0.89(0.04)
	B	0.92	0.86	
	C	0.95	0.86	
0.50	A	0.44	0.42	0.43(0.01)
	B	0.45	0.42	
	C	0.43	0.44	
0.25	A	0.22	0.22	0.22(<0.01)
	B	0.22	0.21	
	C	0.21	0.23	
0.12	A	0.11	0.10	0.11(<0.01)
	B	0.11	0.10	
	C	0.11	0.11	
Solvent Control	A	<0.012	<0.012	
	B	<0.012	<0.012	
	C	<0.012	<0.012	
Control	A	<0.012	<0.012	
	B	<0.012	<0.012	
	C	<0.012	<0.012	
QC#1[b]		0.737 (0.728)[c]	0.547 (0.546)	
QC#2		0.834 (0.819)	0.736 (0.728)	
QC#3		0.913 (0.910)	0.915 (0.910)	

[a] Mean measured values are based on actual unrounded analytical results rather than the rounded numbers presented in this table.

[b] QC = Quality Control

[c] Value in parentheses represents nominal fortified concentration.

Table 3. **Cell density (x 10^4 cells/mL) of *Navicula pelliculosa* after 24, 48, 72, 96 and 120 hours exposure to Thiophanate-methyl .**

Mean Measured Concentration (mg A.I./L)		OBSERVATION INTERVAL (HOURS) 24	48	72	96	120[a]
1.7	A	1	2	3	11	22
	B	0	1	2	14	19
	C	0	1	1	19	19
	Mean(SD)	<1(<1)	1(<1)	2(1)	15(4)	20(2)[b]
0.89	A	1	0	8	20	18
	B	0	1	7	23	26
	C	0	2	7	24	19
	Mean(SD)	<1(<1)	1(1)	7(1)	22(2)	21(5)[b]
0.43	A	1	1	9	18	57
	B	1	1	7	24	49
	C	1	2	8	22	55
	Mean(SD)	1(<1)	1(1)	8(1)	21(3)	54(4)
0.22	A	1	3	6	35	53
	B	2	2	7	31	54
	C	1	3	7	27	61
	Mean(SD)	1(1)	2(1)	6(<1)	31(4)	56(4)
0.11	A	2	5	16	28	49
	B	3	9	20	29	52
	C	2	6	18	30	65
	Mean(SD)	2(1)	7(2)	18(2)	29(1)	55(9)
Solvent Control	A	5	8	15	30	78
	B	3	5	18	32	50
	C	4	7	16	30	50
	Mean(SD)	4(1)	7(2)	16(2)	31(1)	59(16)
Control	A	2	12	13	34	58
	B	6	13	12	38	58
	C	3	12	12	33	50
	Mean(SD)	3(2)	12(<1)	12(1)	35(3)	55(5)
Pooled Control		4(2)	NA	14(3)	33(3)	57(11)

a Cultures were sonicated for five minutes before counting to disperse clumps of cells.
b Significantly reduced ($p < 0.05$) in comparison to pooled control culture (Bonferroni's Test)

navicula cell density

Summary Statistics and ANOVA

Transformation = None

Group (Concentration (mg a.i./l*))	n	Mean	s.d.	cv%
1 = control	6	57.3333	10.8566	18.9
2 0.11	3	55.3333	8.5049	15.4
3 0.22	3	56.0000	4.3589	7.8
4 0.43	3	53.6667	4.1633	7.8
5* 0.89	3	21.0000	4.3589	20.8
6* 1.70	3	20.0000	1.7321	8.7

LOEC = .89
NOEC = 0.43 - however, bloated cells observed at this level.
~~NOEC = 0.22 mg a.i./l*~~
~~LOEC = 0.43 mg a.i./l*~~
not statistically significant

*) the mean for this group is significantly less than the control mean at alpha = 0.05 (1-sided) by a t - test with Bonferroni adjustment of alpha level

* - based on mean recovered concentrations

Minumum detectable difference for
t-tests with Bonferroni adjustment = -11.317691
This difference corresponds to -19.74 percent of control

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**************************************************
*                                                *
* Note - the above value for the minimum         *
* detectable difference is approximate as        *
* the sample sizes are not the same for all of   *
* the groups.                                    *
*                                                *
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Between groups sum of squares = 5410.571429 with 5 degrees of freedom.

Error mean square = 56.711111 with 15 degrees of freedom.

Bartlett's test p-value for equality of variances = .199

MOSSLER THIOPHANATE-METHYL NAVICULA PELLICULOSA 5-1-92

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CONC.	NUMBER EXPOSED	NUMBER DEAD	PERCENT DEAD	BINOMIAL PROB.(PERCENT)
1.7	100	65	65	0
.89	100	63	63	0
.43	100	5	5	0
.22	100	2	2	0
.11	100	4	4	0

BECAUSE THE NUMBER OF ORGANISMS USED WAS SO LARGE, THE 95 PERCENT CONFIDENCE INTERVALS CALCULATED FROM THE BINOMIAL PROBABILITY ARE UNRELIABLE. USE THE INTERVALS CALCULATED BY THE OTHER TESTS.

AN APPROXIMATE LC50 FOR THIS SET OF DATA IS .7743418

RESULTS CALCULATED USING THE MOVING AVERAGE METHOD

SPAN	G	LC50	95 PERCENT CONFIDENCE LIMITS	
2	3.890463E-02	.9338087	.8300291	1.056374

RESULTS CALCULATED USING THE PROBIT METHOD

ITERATIONS	G	H	GOODNESS OF FIT PROBABILITY
4	1.097359	13.86942	0

A PROBABILITY OF 0 MEANS THAT IT IS LESS THAN 0.001.

SINCE THE PROBABILITY IS LESS THAN 0.05, RESULTS CALCULATED USING THE PROBIT METHOD PROBABLY SHOULD NOT BE USED.

SLOPE = 2.493561

95 PERCENT CONFIDENCE LIMITS =-.118566 AND 5.105689

LC50 = 1.016917

95 PERCENT CONFIDENCE LIMITS = .3128021 AND +INFINITY

LC10 = .3147559

95 PERCENT CONFIDENCE LIMITS = 0 AND .6918179

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Shaughnessey # 102001 Chemical Name Thiophanate-methyl Chemical Class _____ Page 1 of 1

Study/Species/Lab/ MRID #	Chemical % a.i.	Results	Reviewer/ Date	Validation Status
14-Day EC_{50}	_____	EC_{50} =. pp 95% C.L. ( ) # plants/vessel = Slope = Species: Temperature = Lab: 14-Day Dose Level pp /(% Effect) ( ), ( ), ( ), ( ), ( ) MRID # Comments:	_____	_____

5-Day EC_{50}	97.6%	EC_{50} = 0.93 ~~pp~~ mg ai/l* 95% C.L. (0.83-1.06) - moving average angle
		 # Cells/ml = 10,000
		 Slope = n/a
		 Species: Navicula pelliculosa
		 Temperature = 25°C
		 Lab: Springborn Laboratories
		 ~~pp~~ mg ai/l*
		 5-Day Dose Level pp /(% Effect)
		 0.11 (4), 0.22 (2), 0.43 (5), 0.89 (63), 1.70 (65)
		 MRID # 42229802
		 Comments:
		 *- based on mean measured concentrations
		 NOEC = 0.43 mg ai/l*
		 LOEC = 0.89 mg ai/l*
			H. Mansfield 3/29/93
			 M. Mossler 5/1/92
				Core